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Intraspecific discrimination power of plastid coding region *rbcL* as DNA barcoding marker in *Dalbergia sissoo* population of Pakistan

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The conservation of diseases resistant sources of *Dalbergia sissoo* germplasm has attained the key status among the Agricultural prospects and solution against their possible threats. For this purpose, the best strategy is DNA barcoding based on nuclear, mitochondrial and plastid genomic regions. This approach is frequently used for species discrimination but its potential to discriminate the individuals at intraspecific level could also be worth exploring considerably. Hence, in the present study the potential of rbcL DNA barcode was evaluated for demonstrating intraspecific variation among the shisham genotypes collected across Pakistan. DNA barcoding analysis was performed on 20 selected shisham genotypes. Nucleotide sequences were analyzed to confirm genetic diversity among studied genotypes ($\theta w = 1.000$, $\pi = 0.00172$). The allelic profile of data yielded 11 unique sequence types (STs) numbers. The phylogenetic tree based on SYM+G model revealed the discriminatory power of rbcL DNA barcode to resolve and differentiate the individuals at intraspecific level.
Keywords: Allelic profile, Sequence types, Akaike Information Criterion, Typing efficiency, DNA substitution.

INTRODUCTION

DNA barcoding is a molecular approach applying to discriminate the individuals at taxonomic level using short stretch of genomic regions. It is a powerful technique to distinguish individuals based on their nucleotide differences in the conserved region of genome. This technique is successfully used for species identification, delimitation and conservation by short universal DNA sequences (Herbert *et al*., 2003; Amaral *et al*., 2004; Hartvig *et al*., 2015). DNA barcoding in animals has been successfully employed, however, in plants; this technique faces different challenges, in terms of genome duplication (nucleus), high chromosomal rearrangement as well as low nucleotide substitution (mitochondria) and slowly evolving genetic regions (plastids). Even then, different DNA regions have been investigated to evaluate their potential as DNA barcodes. In plants, DNA barcodes from nucleus, mitochondrial and plastid regions (*ITS2*, *CO1*, *trnH-psbA, rbcL* and *matK*) have been reported in ecology, systematic, conservation and evolutionary sciences (Kress, 2017). However, the plastid region barcodes are faming great importance towards plant barcoding. Among several plastid regions, two loci namely *rbcL* (ribulose bisphosphate carboxylase large chain) and *matK* (maturase K) are considered to be the potential barcodes for land plants (Hollingsworth *et al*., 2009). These two core loci provide universal framework for several land plants regarding their DNA information. Though in many plant species, the universality and discriminatory power of *rbcL* and *matK* primers remain ~100% and ~35% respectively (Maloukh *et al*., 2017). So, the *rbcL* region has been identified as more important in comparison to *matK* because of its high efficiency, frequency and discrimination power. Hence, in this study, the potential of *rbcl has* been explored to assess its resolution power to discriminate and differentiate the individuals at intraspecific level.

In the present study, the *rbcL* region of *Dalbergia sissoo* (shisham) plantation has been evaluated to understand its discriminatory power at intraspecific level. Because, it is an important timber tree of South-Asia and is being suffered from a great threat of dieback. There is not any cultivar/accession/genotype has identified yet as resistant or susceptible against shisham dieback, although attempts have been made. In our previous studies (Ijaz *et al*., 2018 & 2019),

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we investigated the population genetics based on diversity analysis among 90 shisham genotypes from Pakistan (60 from Punjab, 15 from Sindh, 10 from Khyber Pakhtunkhwa and 5 from Baluchistan). However, diversity study using DNA markers such as RAPD, ISSR, SSR etc, arises different questions that could be addressed by DNA barcoding approach. This study has supported the genetic variation among studied shisham genotypes collected from geographically different regions of Pakistan. Hence, based on the results, rbcL DNA barcode exhibits discriminatory power to resolve the shisham genotypes at intraspecific level.
***Study area*:** Pakistan

MATERIALS AND METHODS

***Molecular and phylogenetic analyses*:** For DNA barcoding 20 dieback disease resistant plants of *Dalbergia sissoo* were used. These were sampled from different regions of Pakistan and grown in the green house of fungal Molecular Biology Laboratory, Department of Plant Pathology, University of Agriculture Faisalabad, Pakistan. The DNA of was isolated from their newly emerging leaves using modified CTAB method by Ijaz *et al*. (2018). In this study, *rbc*L region of chloroplast genome (as DNA barcode) was amplified in the 96-well thermalcycler (peqSTAR) using *rbc*L genetic region based primer pair described by Kress and Erickson (2007) and Fazekas *et al*.(2008). The amplified PCR product was purified using FavorPrep PCR Clean-Up Mini Kit (FAVORGEN Biotech Corp., Taiwan) and sequenced through Eurofins Genomics DNA sequencing services, USA.
The generated sequences were then trimmed by BioEdit sequence alignment editor software and later they were *in silico* characterized by basic local alignment search tool (BLASTn) to check their sequence homology and phylogenetic analysis. These nucleotide sequences were then submitted for GenBank accession numbers. Phylogenetic analysis was performed using MrBayes software to assess the intraspecific variation among these genotypes of Dalbergia sissoo and to evaluate the discriminatory power of rbcL DNA barcode for resolving intraspecific variation. For phylogenetic analysis, sequences were aligned by ClustalW tool and the best-fit model for nucleotide substitution was computed using bioinformatics program MrModeltest2 v.2.3, however, DnaSP v. 6.12.01 was used to calculate nucleotide diversity (π) and polymorphism (θ_w) of the sequences. While allelic profile for 20 sequences was evaluated using MLSTest v. 1.0.

RESULTS

The DNA barcoding approach has potential to discriminate the individuals based on the variation in the nucleotide sequences of the conserved regions in the genome. Therefore, we have attempted to explore the potential of rbcL region of chloroplast genome to evaluate its discriminatory power to resolve the genetic divergence or differences even at intraspecific level. In this study, we have investigated the intraspecific variation among the plants of *Dalbergia sissoo* based on rbcL DNA barcode. The rbcL region of all studied samples was amplified and sequenced based on rbcL DNA barcode based primer pair. The sequences were then deposited to GenBank and were assigned by unique GenBank accession numbers (Table 1). The homology searched by BLASTn showed that they have 100% similarity to already reported *D. sissoo* genotypes. The average GC contents of the sequences were 44.8%.

Table 1. *Dalbergia sissoo* genotypes from different regions of Pakistan, with their plant code and GenBank accession numbers.

Sr.#	Location	Plant code	GenBank accession #
1.	Faisalabad	FP-01	MG462721
2.	Faisalabad	FP-02	MG462722
3.	Bahawalpur	BP-01	MG462723
4.	Rahim Yar Khan	RKP-04-01	MG462724
5.	Mandi Bahauddin	MP-03	MG462725
6.	Khushab	KP-04	MG462726
7.	Bahawalnagar	BNP-03	MG462727
8.	Gujranwala	GP-03	MH023400
9.	Khushab	KP-01	MH023401
10.	Lahore	LP-01	MH023402
11.	Lahore	LP-04	MH023403
12.	Sialkot	SP-01	MH023404
13.	Sialkot	SP-02	MH023405
14.	Toba Tek Singh	TP-04	MH356718
15.	Mandi Bahauddin	MBP-01	MH356719
16.	Khanewal	KWP-03	MH356721
17.	Shorkot	ShP-04	MK163333
18.	Okara	OP-02	MK163334
19.	Chichawatni	CP-01	MK163335
20.	Khanewal	KWP-04	MK163336

The nucleotide polymorphism, θ_w, and nucleotide diversity, π, of *rbcL* region of these genotypes was estimated as 1.000 and 0.00172 respectively. The θ_w was based on Watterson's estimator (1975) while π was calculated accordance to Nei and Li (1979). While, their allelic profile suggested 11 unique sequence types (STs) numbers along with their frequency in genotypes (Table 2& 3). The number of polymorphism, typing efficience (TE) and discriminatory power (DP) by Simpson's index (with 95% CI) were recorded as 80, 0.138 and 0.805 (0.6-1) respectively. The delta score based on distance data was 0.03158 on an average, however delta score

of each genotype with sequrnce types (STs) numbers given in Table 2.

Table 2. A list of Delta scores and STs for individual genotype.

Shisham genotypes	Delta Score	Sequence Types (STs)
MBP-01	0.017544	1
SP-02	0.015789	2
BNP-03	0.017544	3
MP-03	0.017544	3
RKP-04-01	0.017544	4
FP-01	0.017544	5
FP-02	0.017544	5
BP-01	0.015789	6
KP-04	0.017544	5
GP-03	0.017544	5
KP-01	0.017544	7
LP-01	0.017544	5
LP-04	0.017544	5
SP-01	0.017544	8
TP-04	0.017544	9
KWP-03	0.017544	10
Shp-04	0.017544	5
OP-02	0.017544	11
CP-01	0.017544	5
KWP-04	0.017544	5

Table 3. Sequence types (STs) numbers and their frequency in genotypes

STs	Frequency	Genotype
1	1	MH356719_MBP-01
2	1	MH023405_SP-02
3	2	MG462727_BNP-03, MG462725_MP-03
4	1	MG462724_RKP-04-01
5	9	MG46272_FP-01, MG462722_FP-02, MG462726_KP-04, MH023400_GP-03, MH023402_LP-01, MH023403_LP-04, MK163333_Shp-04, MK163335_CP-01, MK163336_KWP-04
6	1	MG462723_BP-01
7	1	MH023401_KP-01
8	1	MH023404_SP-01
9	1	MH356718_TP-04
10	1	MH356721_KWP-03
11	1	MK163334_OP-02

For Phylogenetic analysis to analyze the discriminatory power of rbcL DNA barcode region, MrModeltest2 suggested SYM+G as best-fit model of Akaike Information Criterion (AIC) for DNA substitution of the nucleotide data. This suggested model (SYM+G) was used to assess phylogenetic relationship among *D. sissoo* genotypes with MrBayes software. This bayesian analysis comprised of four chains of MCMC (Markov chain Monte Carlo) with random starting trees. These MCMC chains were run for 2×10^6 generations with sample frequency 1000 at the rate of 0.25 burnin frequency. The consensus tree was constructed based on 50% majority rule. In phylogenetic tree, pharaphyletic grouping of the collected *D. sissoo* genotypes were observed with maximum of 20% bootstrap support (Fig. 1). The MP-03 genotype observed as distinct one that was rooting the whole tree, however, it showed genetic relationship with BP-01 and KWP-03 genotypes with only 20% bootstrap support. While BP-01 and KWP-03 were close to each other with 18% bootstrap support. Though, in the tree OP-02 and SP-02 genotypes were observed in close relation but with 2% bootstrap support only. These results unraverled the potential of rbcL DNA barcode to discriminate the indiuals at intraspecific level

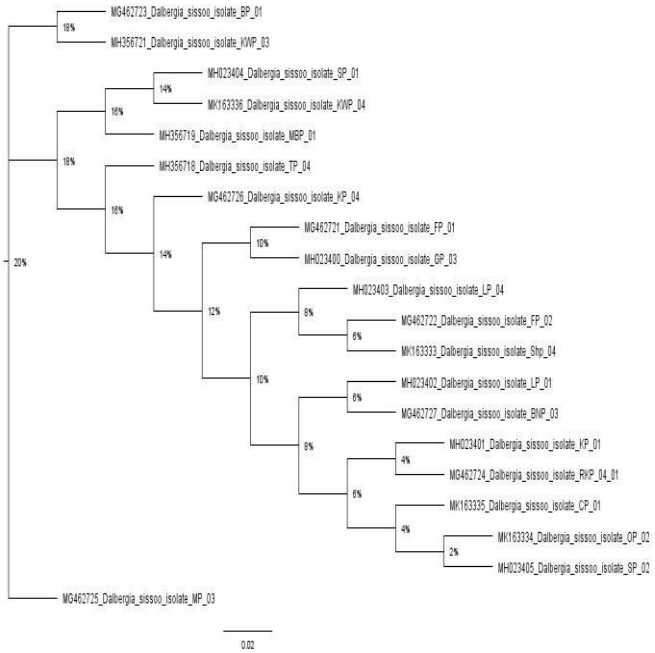

Figure 1. Phylogenetic tree based on rbcL region of *Dalbergia sissoo* genotypes collected across Pakistan.

DISCUSSION

DNA barcoding is remarkable approach for assessing the evolutionary variation among or within species. To date, this approach is widely used in plants on their conserved genomic regions of nucleus, chloroplast and mitochondria. Among others, plastid based *rbcL* region showed high potential, universality and discriminatory power (Bhagwat *et al*., 2015). In the present study, diversity of twenty shisham genotypes from different regions of Pakistan was resolved using *rbcL* marker. The results were proved to be milestone toward *rbcL* resolving power and its potential to discriminate individual at

intraspecific level. Sequence based genetic diversity of shisham genotypes confirmed the results of Ijaz *et al*. (2018, 2019) in more precise manner and supported the molecular genotyping for population genetics.

In this study, nucleotide sequence analysis was performed with DnaSP that showed the genetic relationship among collected genotypes (θ_w = 1.000, π = 0.00172, Delta score = 0.03158). However, their allelic profile based on *rbcL* gene supported strongly the presence genetic difference among these genotypes by revealing 11 good sequence types (STs) numbers. The pattern of cladogram in phylogenetic tree based on rbcL barcoding marker also showed similarity with dendrogram reported for genetic diversity of shisham based on ISSR marker (Ijaz *et al*., 2018). Thus, the overall results confirmed the strength of *rbcL* DNA barcoding marker to differentiate and discriminate the shisham genotypes at intraspecific level. This DNA barcoding marker revealed the intraspecific variation among the shisham genotypes to resolve their genetic difference.

***Conclusion*:** With the potential use of DNA barcoding, assessment of genotypic relationship among different individual has become an easy task. This study presents unequivocal genotyping and the relationship among shisham genotypes in Pakistan. The discriminatory power of *rbcL* DNA barcoding marker has enabled us in a more precise way to resolve the diverse and scattered population of *Dalbergia sissoo* at intraspecific level. This step will also help in tagging and organizing the shisham population of Pakistan.

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***Ethical statement*:** This article does not contain any studies with human participants or animal performed by any of the authors.

***Availability of data and material*:** I declare that the submitted manuscript is our own work, which has not been published before and even is not currently being considered for publication elsewhere. And there is no conflict of interest.

Code Availability: Not applicable

***Consent to participate*:** Siddra Ijaz and Imran Ul Haq designed and developed the experiment. Mubashir Abbas and Muhammad Asif Sakhawat experimented. Siddra Ijaz, Imran Ul Haq, Hafiza Arooj Razzaq, Bukhtawer Nasir, Maria Babar, Mubashir Abbas and Muhammad Asif Sakhawat analyzed and interpreted the data, drafted and revised the manuscript.

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REFERENCES

Amaral, W., E.D. Kjaer, A. Yanchuk and L. Graudal. 2004. Research needs. FOREST genetic resources conservation and management: overview, concepts and some systematic approaches. Rome: International Plant Genetic Resources Institute 1:37-47.

Bhagwat, R.M., B.B. Dholakia, N.Y. Kadoo, M. Balasundaran and V.S. Gupta. 2015. Two new potential barcodes to discriminate Dalbergia species. PloS one 10: e0142965.

Fazekas, A.J., P.R. Kesanakurti, K.S. Burgess, D.M. Percy, S.W. Graham, S.C. Barrett, S.G. Newmaster, M. Hajibabaei and B.C. Husband. 2009. Are plant species inherently harder to discriminate than animal species using DNA barcoding markers? Molecular Ecology Resources 9:130-139.

Hartvig, I., M. Czako, E.D. Kjær, L.R. Nielsen and I. Theilade. 2015. The use of DNA barcoding in identification and conservation of rosewood (Dalbergia spp.). PLoS One 10:e0138231.

Hebert, P.D., A. Cywinska, S.L. Ball and J.R. Dewaard. 2003. Biological identifications through DNA barcodes. Proceedings of the Royal Society of London. Series B: Biological Sciences 270:313-321.

Hollingsworth, P.M., L.L. Forrest, J.L. Spouge, M. Hajibabaei, S. Ratnasingham, M. van der Bank, M.W. Chase, R.S. Cowan, D.L. Erickson and A.J. Fazekas. 2009. A DNA barcode for land plants. Proceedings of the National Academy of Sciences 106:12794-12797.

Ijaz, S., I. Haq, H.A. Razzaq, B. Nasir, B and M. Babar. 2019. ISSR based population genetics study for tagging a diverse population of Shisham (Dalbergia sissoo) in Pakistan. Applied Ecology and Environmental Research 17:5851-5861.

Ijaz, S., H.A. Razzaq, M. Babar and I. Haq. 2018. Assessment of population genetics of shisham (Dalbergia sissoo) based on genetic structure and diversity analysis. International Journal of Biosciences 13:209-222.

Kress, W.J. 2017. Plant DNA barcodes: Applications today and in the future. Journal of systematics and evolution 55:291-307.

Kress, W. J. and D.L. Erickson. 2007. A Two-Locus Global DNA Barcode for Land Plants: The Coding rbcL Gene Complements the Non-Coding trnH-psbA Spacer Region. Plos One 2:e508.

Maloukh, L., A. Kumarappan, M. Jarrar, J. Salehi, H. El-Wakil and T.R. Lakshmi. 2017. Discriminatory power of

rbcL barcode locus for authentication of some of United Arab Emirates (UAE) native plants. 3 Biotech 7:144.

Nei, M. and W.H. Li. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proceedings of the National Academy of Sciences 76:5269-5273.

Tomasini, N., J.J. Lauthier, M.S. Llewellyn and P. Diosque. 2013. MLSTest: novel software for multi-locus sequence data analysis in eukaryotic organisms. Infection, Genetics and Evolution 20:188-196.

Watterson, G.A. 1975. On the number of segregating sites in genetical models without recombination. Theoretical population biology 7:256-276.